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**WO 00/76539 A2**

(54) Title: **A METHOD TO IMPROVE PROTECTIVE IMMUNITY INDUCED BY POLYNUCLEOTIDE VACCINES**

(57) Abstract: A method of enhancing protective immunity induced by any polynucleotide vaccine or combination of polynucleotide vaccines comprising immunizing with a priming immunization mixture of a polynucleotide vaccine mixed with a polynucleotide vector that expresses a cytokine. Subsequently the priming vaccination is followed by immunizing with a boosting immunization preparation that contains a recombinant pox virus. This method also reduces the amount of vaccine needed to achieve protective immunity and broadens the coverage in a vaccinated population.

## A METHOD TO IMPROVE IMMUNITY INDUCED BY POLYNUCLEOTIDE VACCINES

### 5 *Field of the Invention*

This invention relates to the prophylaxis of, and treatment for, malaria (*Plasmodium falciparum*, *P. vivax*, *P. malariae*, *P. ovale*) and other diseases by providing a method to improve immunity induced by polynucleotide vaccines.

10 Malaria is a public health problem in more than 90 countries, inhabited by a total of some 2.4 billion people or 40% of the world's population. Worldwide incidence of the disease is estimated to be on the order of 300-500 million clinical cases each year. Mortality due to malaria is estimated to be in the range of 1.5 to 2.7 million deaths annually. The malaria parasite has become resistant to antimalarial drugs in many parts of the world and the incidence of drug resistance continues to increase. An acceptable vaccine to prevent malaria is not currently  
15 available although protection against a well-controlled malaria challenge has been achieved experimentally in small numbers of human subjects by an extensive course of immunization with live, irradiation-attenuated parasites. The emerging technology of polynucleotide vaccines, also termed DNA vaccines by Donnelly (Ann. Rev. Immun. 15:617-648, 1997), offers a practical alternative method for the development of a multivalent vaccine capable of inducing the panoply  
20 of protective immune responses that are induced by the attenuated parasite vaccine. However, in order to achieve optimal immunity, the polynucleotide dosage levels that are being administered in these experiments are much too high. The costs associated with pharmaceutical manufacturing at these high levels are prohibitively expensive. Furthermore, at these levels the potential for adverse effects in vaccine recipients is increased.

25 A polynucleotide vaccine based on the malaria circumsporozoite protein (CSP) gene from

*Plasmodium yoelii* (PyCSP) has undergone extensive experimental evaluation in the rodent malaria model system by Sedegah et al. (Proc. Natl. Acad. Sci. USA 91:9866-9870, 1994), Doolan et al. (J. Exp. Med. 183:1739-1746, 1996), and Sedegah et al. (Proc. Natl. Acad. Sci. USA 95:7648-7653, 1998) and recently the first clinical trial to evaluate the safety and immunogenicity of a *Plasmodium falciparum* (Pf) CSP polynucleotide vaccine (pPfCSP) in healthy volunteers was concluded by Wang et al. (Science 282:476-480, 1998). In the rodent malaria model system, IM immunization with the polynucleotide vaccine for PyCSP (pPyCSP) induces an excellent antibody response and the highest CD8+ CTL responses that we have observed with any vaccine preparation available. However, this immunization is not optimal for protection because, unlike the irradiation-attenuated parasite vaccine, 100% protective efficacy was not achieved. Instead, protection levels range between 25% to 75%, depending on the number of doses given, dose intervals, and infectivity of the sporozoite challenge. Efforts to improve protective efficacy with the polynucleotide vaccine led to experiments in which a dosing regime, referred to as prime/boost, was shown to induce higher levels of antibodies, CTL responses, and protection. As shown by Sedegah et al. (Proc. Natl. Acad. Sci. USA 95:7648-7653, 1998), priming with DNA and boosting with recombinant vaccinia virus proved to be better at inducing protection than priming and boosting with the pPyCSP alone. In another series of experiments aimed at improving protective efficacy, Weiss et al. (J. Immunol. 161:2325-2332, 1998) showed that immunization of mice with two doses at 6 week intervals of a mixture of pPyCSP and a plasmid expressing murine granulocyte macrophage colony stimulating factor (pGMCSF) gave a better protective immune response than immunization with pPyCSP alone.

In summary, polynucleotide vaccines for malaria and other applications offer the potential for providing multivalent preventatives that are safe and practical. One disadvantage that

currently limits this potential, however, is that excessively high doses are required in order to achieve protective efficacy.

#### SUMMARY OF THE INVENTION

Accordingly, an object of this invention is a method of enhancing protective immunity  
5 induced by any polynucleotide vaccine.

Another object of this invention is a method of conserving or reducing the amount of DNA vaccine needed to induce protection.

A further object of the invention is a recombinant GMCSF expression system.

An additional object of the invention is the use of a plasmid expressing GMCSF to  
10 enhance the immunogenicity of *PyCSP* and *PfCSP*.

Yet an additional object of the invention is an immunization regime whereby recombined *PyCSP* and *PfCSP* is used to enhance the immunogenicity of *PyCSP* and *PfCSP*.

These and additional objects of the invention are accomplished by a method of enhancing protective immunity induced by any polynucleotide vaccine or combination of polynucleotide  
15 vaccines comprising immunizing with a priming immunization mixture of a polynucleotide vaccine mixed with a polynucleotide vector that expresses a cytokine. Subsequently the priming vaccination is followed by immunizing with a boosting immunization preparation that contains a recombinant pox virus. This method also reduces the amount of vaccine needed to achieve protective immunity and broadens the coverage in a vaccinated population.

#### DESCRIPTION OF THE PREFERRED EMBODIMENTS

20 The widespread deployment of polynucleotide vaccines may depend upon the use of immunization regimes in which the vaccine is administered together with cytokine expressing plasmids and recombinant pox viruses (canarypox, cowpox, fowlpox, adenovirus, adeno-

associated virus, alphaviruses [replicons of Semliki Forest virus and Sindbis virus] for instance, and others like them). We have determined that the inclusion of a cytokine plasmid (these include lymphokines such as the interleukins, tumor necrosis factor and the interferons; growth factors such as granulocyte-macrophage colony stimulating factor) during priming with a polynucleotide vaccine followed approximately three (3) or more days later by a booster immunization with recombinant vaccinia viruses, provides a method of immunization that induces an enhanced immunogenic response to the encoded antigen and a greater level of protective immunity than by priming with the polynucleotide vaccine alone. As a result of this method, the dose of polynucleotide vaccine needed for efficacy may be reduced by several orders of magnitude.

In the *P. yoelii* rodent malaria model, PyCSP is a target of protective antibodies, CD4+ T cells and protective CD8+ T cells. Therefore, the pPyCSP polynucleotide vaccine in mice provided an ideal experimental system with which to measure the enhancing effect of priming and boosting a polynucleotide vaccine with, respectively, a GM-CSF expression plasmid (pGMCSF) and a recombinant pox virus expressing the vaccine antigen (rvPyCSP). A suboptimal dosing regime was employed that allowed enhancement to be measured more readily. The regime consisted of the priming immunization followed 3 weeks later by the boost. The different regimes are depicted in the specific examples.

Having described the invention, the following examples are given to illustrate specific applications of the invention including the best mode now known to perform the invention. These specific examples are not intended to limit the scope of the invention described in this application.

### **EXAMPLES**

In 3 separate experiments, homologous priming and boosting with high DNA dose, 100µg of PyCSP DNA (DD) or low DNA dose, 1µg of PyCSP DNA (d/d); or homologous priming and

boosting with high DNA dose mixtures of 100 $\mu$ g of PyCSP DNA and 30 $\mu$ g of GMCSF DNA (DG/DG) or low DNA dose mixtures of 1 $\mu$ g of PyCSP DNA and 1 $\mu$ g of GMCSF DNA (dg/dg) did not lead to significant protection. However heterologous prime/boost immunizations involving priming with mixtures of PyCSP and GMCSF DNAs even with the lower doses resulted in higher level of protection.

**Example 1:** In Experiment 1, mice primed and boosted with 100 $\mu$ g of PyCSP DNA (D/D) given at 3 week interval gave minimal protection, (1/8), but was increased (3/8) when mice were primed and boosted with a mixture of 100 $\mu$ g of PyCSP DNA and 30 $\mu$ g of GMCSF DNA (DG/DG). When mice were primed and boosted with 1 $\mu$ g of PyCSP DNA (d/d), no mouse was protected, (0/8), and when mice were primed and boosted with a mixture of 1 $\mu$ g of PyCSP DNA and 1 $\mu$ g of GMCSF DNA (dg/dg), minimal protection, (1/8), was seen. All mice were challenged with 50 sporozoites two weeks after boost and monitored for parasitaemia in a 14 day follow up period.

**Example 2:** In Experiment 2, mice primed and boosted with 100 $\mu$ g of PyCSP DNA (D/D) given at 3 week interval gave no protection, 0/10, and was hardly increased (1/10) when mice were primed and boosted with a mixture of 100 $\mu$ g of PyCSP DNA and 30 $\mu$ g of GMCSF DNA (DG/DG). However when mice were primed with 100 $\mu$ g of PyCSP DNA and boosted with  $1 \times 10^7$  pfu of recombinant vaccinia expressing PyCSP (D/V), or primed with a mixture of 100 $\mu$ g of PyCSP DNA and 30 $\mu$ g of GMCSF DNA and boosted with  $1 \times 10^7$  pfu of recombinant vaccinia expressing PyCSP (DG/V), 4 out of 10, and 8 out of 10 respectively were protected. All mice were challenged with 50 sporozoites two weeks after boost and monitored for parasitaemia in a 14 day follow up period.

**Example 3:** In Experiment 3, mice primed and boosted with 100 $\mu$ g of PyCSP DNA (D/D)

given at 3 week interval protected 2 of 8 mice, but 7 of 8 mice were protected when mice were primed with a mixture of 100 $\mu$ g of PyCSP DNA and 30 $\mu$ g of GMCSF DNA and boosted with  $2 \times 10^7$  pfu of recombinant vaccinia expressing PyCSP (DG/V). The most striking finding however was that when priming was done with low dose DNA mixture made up of 1 $\mu$ g of PyCSP DNA (100 times lower) and 1 $\mu$ g of GMCSF DNA and boosted with  $2 \times 10^7$  pfu of recombinant vaccinia expressing PyCSP (dg/V), a high level of protection, 7 of 8 mice were protected. Protection was due to the PyCSP DNA since priming with a high dose DNA mixture consisting of 100 $\mu$ g of empty plasmid DNA and 30 $\mu$ g of GMCSF DNA and boosted with  $2 \times 10^7$  pfu of recombinant vaccinia expressing PyCSP (CG/V) did not lead to significant protection (1 of 8 mice). All mice were challenged two weeks after boost with 50 sporozoites and monitored for parasitaemia in a 14 day follow up period.

**Results:** The results indicate that when pGMCSF is included in the priming, not only is the immunogenicity and protection increased, but also the amount of PyCSP DNA required could be reduced by at least a hundredfold. Combined data from Examples 1, 2 and 3 (Table 1) of experiments conducted with two immunizations at the suboptimal regimen of three week intervals show that the DG-V regimen was the most. Combinin results of experiments 1, 2 & 3, a total of 15 out of 18 (83.3 %) challenged DG-V mice were protected, compared to 4 out of 18 (22.2%) of the DG-DG and to 3 out of 26 (11.5%) of the D-D. Low dose priming with plasmid mixtures (dg-V) was more protective than DG-DG, D-D regimen and in one experiment, the DG-V or dg-V protected mice to the same degree, 7 out of 8 (87.5%).

Further support that low dose pGMCSF in the prime/boost regimen enables enhanced immunity is seen in the data shown in Tables 2 and 3. High levels of antibodies against sporozoites were detected in the highly protected groups. We noted a higher level of protective

antibodies due to the priming with mixture of pPyCSP and pGMCSF followed by boosting with a  
 rvPyCSP. In Table 3 it can be seen that the groups producing the highest levels of antibody were  
 those in which the pGMCSF prime/boost regimen was employed. Furthermore, the low dose  
 regimen (dg-V) elaborated levels of antibodies in the mice that were surpassed only by the high  
 5 dose regimen (DG-V). In fact these two prime/boost groups appeared to induce equivalent levels  
 of antibody against the intact parasite as measured by IFA titer.

The data presented demonstrates that priming with a mixture of PyCSP and GMCSF  
 plasmid DNA induces higher levels of protective immunity than does priming with PyCSP without  
 GMCSF. In previous studies, we have shown that, in order of increasing protective efficacy, DG-  
 10 DG was greater than D-D, and D-V was greater than D-D or V-V. We now show that D-V is  
 better than DG-DG, and DG-V is better than D-V. Furthermore, by including GMCSF, the  
 amount of DNA required to achieve high levels of protection can be reduced by a hundredfold  
 and ongoing experiments varying the experimental conditions may demonstrate that this can be  
 reduced even further. This ability to reduce the amount of vaccine needed to provide efficacy  
 15 confers a significant new advantage for polynucleotide vaccines.

**Table 1. Addition of plasmid expressing GMCSF increases protection after boost.**

<b>Prime</b>	<b>Boost</b>	<b>Protected/Total</b>	<b>% Protection</b>
<b>Experiment 1</b>			
<b>D</b>	<b>D</b>	<b>1/8</b>	<b>12.5%</b>
<b>DG</b>	<b>DG</b>	<b>3/8</b>	<b>37.5%</b>
<b>D</b>	<b>d</b>	<b>0/8</b>	<b>0</b>
<b>Dg</b>	<b>dg</b>	<b>1/8</b>	<b>12.5%</b>
<b>Naïve</b>	<b>-</b>	<b>0/10</b>	<b>0</b>



**Experiment 2**

D	D	0/10	0
DG	DG	1/10	10%
D	V	4/10	40%
DG	V	8/10	80%
CG	V	0/10	0
Naïve	-	0/10	0

**Experiment 3**

D	D	2/8	25%
DG	V	7/8	87.5%
Dg	V	7/8	87.5%
CG	V	1/8	12.5%
Naïve	-	0/8	0

**Table 2. Addition of GMCSF expressing plasmid during priming: Effect on antibody response, inhibition of liver stage development, and protection.**

Prime	Boost	IFA Titer	% Protection	% Inhibition of Liver stage development (ILSDA)
D	D	10,240	25	45
DG	V	162,840	87.5	73
dg	V	81,920	87.5	58
CG	V	2,560	12.5	20

Naive	-	<10	0	-
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Pre-challenge pooled sera from mice primed and boosted in Experiment 3 (described above in Table 3), were evaluated for anti *Py* sporozoite antibody titres on sporozoites. Diluted sera were added to air dried sporozoites and the highest serum dilution to give fluorescence was scored as a titre. Results of in vitro inhibitory effects of restimulated spleen cells from the different groups on the development of liver stage parasites, as well as sterile protection results are compared with the antibody titres.

**Table 3. Induction of antibodies to intact sporozoites, a recombinant polypeptide Py CSP and a synthetic peptide of the protective B-cell epitope of PyCSP.**

Prime	Boost	IFA Titer	ELISA OD <sub>0.5</sub> (units)	
			rPyCSP	(QGPGAP) <sub>4</sub>
DG	DG	20,480	26,667	1,800
D	V	40,960	64,000	1,333
DG	V	81,920	192,000	6,000
CG	V	640	1,000	neg
dg	dg	5,120	14,400	neg
d	V	10,240	6,000	neg
dg	V	81,920	85,333	1,800

cg	V	1,280	800	neg
Naive	-	<10	0	-

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In this experiment, two high and low dose groups of three prime/boost combinations were compared. In the high dose DNA group, mice were primed and boosted with a mixture of 100 $\mu$ g of PyCSP DNA and 30 $\mu$ g of GMCSF DNA (DG/DG); primed with 100 $\mu$ g of PyCSP DNA and boosted with 2 x 10<sup>7</sup> pfu of recombinant vaccinia expressing PyCSP (D/V); primed with a mixture of 100 $\mu$ g of PyCSP DNA and 30 $\mu$ g of GMCSF DNA and boosted with 2 x 10<sup>7</sup> pfu of recombinant vaccinia expressing PyCSP (DG/V) and a control group was primed with a mixture of 100 $\mu$ g of empty plasmid and 30 $\mu$ g of GMCSF DNA and boosted with 2 x 10<sup>7</sup> pfu of recombinant vaccinia expressing PyCSP (CG/V). In the second set, low dose DNA was used and mice were primed and boosted with a mixture of 1 $\mu$ g of PyCSP DNA and 1 $\mu$ g of GMCSF DNA (dg/dg); primed with 1 $\mu$ g of PyCSP DNA and boosted with 2 x 10<sup>7</sup> pfu of recombinant vaccinia expressing PyCSP (d/V); primed with a mixture of 1 $\mu$ g of PyCSP DNA and 1 $\mu$ g of GMCSF DNA and boosted with 2 x 10<sup>7</sup> pfu of recombinant vaccinia expressing PyCSP (dg/V); and a control group was primed with a mixture of 1 $\mu$ g of empty plasmid and 1 $\mu$ g of GMCSF DNA and boosted with 2 x 10<sup>7</sup> pfu of recombinant vaccinia expressing PyCSP (cg/V). The antibody titres to whole sporozoites as well as the titres to the protective B-cell epitope or recombinant PyCSP protein are compared. Results show that in both high and low PyCSP DNA dose regimens, IFA antibody levels produced to sporozoites in order of highest reactivity was DG/V>D/V>DG/DG>CG/V. In all, the DG/V and dg/V regimen produced the highest titers to either the recombinant protein or the protective PyCSP B-cell epitope, (QGPGAP).

Obviously, many modifications and variations of the present invention are possible in light of the above teachings. It is therefore to be understood that, within the scope of the appended claims, the invention may be practiced otherwise than as specifically described.

What is claimed is:

1. A method to enhance protective immunity induced by any polynucleotide vaccine or combination of polynucleotide vaccines comprising;
  - a. immunizing with a priming immunization mixture in which a polynucleotide vaccine is mixed with a polynucleotide vector that expresses a cytokine followed by,
  - b. immunizing subsequently with a boosting immunization preparation in which;
    - 1) any recombinant virus expression system is in the preparation or,
    - 2) immunizing subsequently with a boosting immunization preparation in which any recombinant protein antigen is in the preparation.
2. The method of Claim 1, wherein said expression system is a recombinant pox virus.
3. The method of Claim 1, wherein said expression system is selected from the group consisting of cowpox, canarypox, fowlpox, adenovirus, adenoassociated virus, alpha virus, Semiliki Forest, and Sindbis virus.
4. The method of Claim 1, wherein said expression system is a recombinant protein antigen..
5. The method of Claim 1, wherein said recombinant protein antigen is a neoplastic tumor.
6. The method of Claim 1, wherein said recombinant protein antigen is rvPyCSP.
7. The method of Claim 1, wherein said polynucleotide vector is a recombinant plasmid.
8. The method of Claim 1, wherein said cytokine is selected from the group consisting of GMCSF, the interleukins, tumor necrosis factor, and the interferons.
10. The method of Claim 1, wherein said polynucleotide vaccine comprises a malaria PyCSP gene.

11. The method of Claim 1, wherein said polynucleotide vaccine comprises a PfCSP gene.
12. A method to reduce the amount of vaccine required to achieve protective immunity induced by any polynucleotide vaccine or combination of polynucleotide vaccines comprising;
- 5 a. immunizing with a priming immunization mixture in which polynucleotide vaccine is mixed with a polynucleotide vector that expresses a cytokine followed by,
- b. immunizing subsequently with a boosting immunization preparation in which ;
- 1) any recombinant virus expression system is in the preparation or,
- 10 2) immunizing subsequently with a boosting immunization preparation in which any recombinant protein antigen is in the preparation.
13. The method of Claim 12, wherein said expression system is a recombinant pox virus.
14. The method of Claim 12, wherein said expression system is selected from the group
- 15 consisting of cowpox, canarypox, fowlpox, adenovirus, adenoassociated virus, alpha virus, Semiliki Forest, and Sindbis virus.
15. The method of Claim 12, wherein said recombinant protein antigen is an infectious disease agent.
16. The method of Claim 12, wherein said recombinant protein antigen is a neoplastic tumor.
- 20 17. The method of Claim 12, wherein said recombinant protein antigen is rvPyCSP.
18. The method of Claim 12, wherein said polynucleotide vector is a recombinant plasmid.
19. The method of Claim 12, wherein said cytokine is selected from the group consisting of GMCSF, the interleukins, tumor necrosis factor, and the interferons.

20. The method of Claim 12, wherein said polynucleotide vaccine comprises a malaria PyCSP gene.
21. The method of Claim 12, wherein said polynucleotide vaccine comprises a PfCSP gene.
22. A method to broaden the coverage in a vaccinated population of the protective efficacy  
5 induced by any polynucleotide vaccine or combination of polynucleotide vaccines comprising;
- a. immunizing with a priming immunization mixture in which polynucleotide vaccine is mixed with a polynucleotide vector that expresses a cytokine followed by,
  - b. immunizing subsequently with a boosting immunization preparation in  
10 which ;
- 1) any recombinant virus expression system is in the preparation or,
  - 2) immunizing subsequently with a boosting immunization preparation  
in which any recombinant protein antigen is in the preparation.
23. The method of Claim 22, wherein said expression system is a recombinant pox virus.
- 15 24. The method of Claim 22, wherein said expression system is selected from the group consisting of cowpox, canarypox, fowlpox, adenovirus, adenoassociated virus, alpha virus, Semiliki Forest, and Sindbis virus.
25. The method of Claim 22, wherein said recombinant protein antigen is an infectious disease agent.
- 20 26. The method of Claim 22, wherein said recombinant protein antigen is a neoplastic tumor.
27. The method of Claim 22, wherein said recombinant protein antigen is rvPyCSP.
28. The method of Claim 22, wherein said polynucleotide vector is a recombinant plasmid.
29. The method of Claim 22, wherein said cytokine is selected from the group consisting of

GMCSF, the interleukins, tumor necrosis factor, and the interferons.

30. The method of Claim 22, wherein said polynucleotide vaccine comprises a malaria PyCSP gene.

31. The method of Claim 22, wherein said polynucleotide vaccine comprises a PfCSP gene.



## Hemby, Joseph

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**From:** Hemby, Joseph  
**Sent:** Wednesday, April 09, 2008 4:20 PM  
**To:** Schlagel, Charles GS NMRC  
**Cc:** Simmons, Rita LCDR; Hemby, Joseph  
**Subject:** FW: CRADA  
**Importance:** High  
**Attachments:** Capable Manpower-Clemson CRADA 01APRIL2008.doc

Charlie,

I see only one real issue which is Clemson wants the laws of South Carolina to govern the agreement. That has to be changed back to the Federal laws.

Thanks,

Ken

---

**From:** Simmons, Rita G. - CDR  
**Sent:** Wednesday, April 09, 2008 1:01 PM  
**To:** Hemby, Joseph  
**Subject:** FW: CRADA  
**Importance:** High

Mr. Hemby,  
Sorry for the confusion, but the NMRC limited CRADA you have is ours. It appears that NMRC was accidentally left on the document. I am attaching a copy with NAMRL in the title instead of NMRC. Please let me know if you have any other questions.  
Thank you for your quick response.  
CDR Simmons

CDR Rita G. Simmons, MSC, USN  
Officer in Charge, Acting  
Naval Aerospace Medical Research Laboratory  
280 Fred Bauer St.  
NAS Pensacola, FL 32508  
850-452-3573  
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---

**From:** Schoenberg, Larry - CTR  
**Sent:** Wednesday, April 09, 2008 12:56 PM  
**To:** Simmons, Rita G. - CDR  
**Subject:** FW: CRADA

CDR S.

Please forward to Ken for review.

Vr,

4/9/2008

Larry

Larry Schoenberg, Contractor  
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---

**From:** Schoenberg, Larry - CTR  
**Sent:** Monday, April 07, 2008 2:31 PM  
**To:** Schlagel, Charles GS NMRC  
**Cc:** Phillips, Jeffrey B. - CIV  
**Subject:** FW: CRADA

Charlie,

Just checking to make sure you got this. To clarify, the Clemson changes are the only ones with comments.

Thanks,

Larry

Larry Schoenberg, Contractor  
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---

**From:** Schoenberg, Larry - CTR  
**Sent:** Tuesday, April 01, 2008 2:26 PM  
**To:** Schlagel, Charles GS NMRC  
**Cc:** Phillips, Jeffrey B. - CIV  
**Subject:** FW: CRADA

Charlie,

Here is draft CRADA. Please assign CRADA number.

Clemson changes are tracked.

If changes to liability and governing law are unacceptable, please suggest alternative solution to provide to Clemson. If direct liaison between you and Clemson legal is necessary to clarify issues, can you contact legal POC listed in CRADA?

Thanks,

Larry

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---

**From:** Phillips, Jeffrey B. - CIV  
**Sent:** Tuesday, April 01, 2008 11:22 AM  
**To:** Schoenberg, Larry - CTR  
**Subject:** FW: CRADA

Mr. Schoenberg,

Here is the CRADA back from the Clemson lawyers. It looks as if they only had issues with the liability section. There are also some changes that we have since made regarding the specific materials to be transferred.

Thanks,  
Jeff

---

**From:** Strompolis, Melissa E. - CTR  
**Sent:** Tuesday, April 01, 2008 8:02 AM  
**To:** Phillips, Jeffrey B. - CIV  
**Subject:** CRADA

CRADA with Clemson edits and all items to be delivered.

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4/9/2008